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Therapeutic use of fluorinated nucleosides – progress in patents

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Abstract

Fluorinated nucleosides constitute a large class of chemotherapeutics approved for clinical use. The pharmacokinetic and pharmacodynamic properties of a drug can be affected, as a consequence of modulation of electronic, lipophilic and steric parameters, by the introduction of fluorine into the structure of drug-like molecule. Herein, we focus on fluorinated-nucleoside analogs, their therapeutic use and applications based on the patent literature from 2014 to 2018. We briefly discuss the clinical properties of anticancer and antiviral fluorine-containing nucleos(t)ides FDA-approved or in development, and highlight their resistance mechanisms and limitations in the clinic. We emphasize patent inventions related to improved synthetic methods towards selected nucleos(t)ide analogs including the phosphoramidate sofosbuvir and ¹⁸F-labelled nucleosides FLT and FMAU, used as a ¹⁸F-PET tracers.

Keywords: Fluorinated-nucleosides, phosph(on)ate prodrugs, anticancer, antiviral, N-glycosylation, nucleophilic fluorination, ¹⁸F-PET tracers.

Introduction

Fluorine organic chemistry plays a significant role in a number of different research fields. During the last three decades, fluoro-organic chemistry has become a research area of high interest, in particular for several different drug discovery programs. Fluorine insertion in the structures of bioactive organic molecules has effects on several molecular properties such as electronics, occupational volume and lipophilicity, this way affecting pharmacodynamic and pharmacokinetic features of drug-like compounds. Hydrogen replacement with fluorine can remove a potential oxidative site for drug metabolism, thus avoiding the production of unwanted metabolites [1,2].

Fluorine also has a very important function in positron emission tomography (PET), thanks to the favourable, relatively short half-life of its radioisotope ¹⁸F (109.8 min), which makes possible to synthesise the tracer and carry out the imaging process within few hours. Moreover, the use of ¹⁸F is advantageous for imaging, as it gives good spatial resolution, due to its nature of low positron

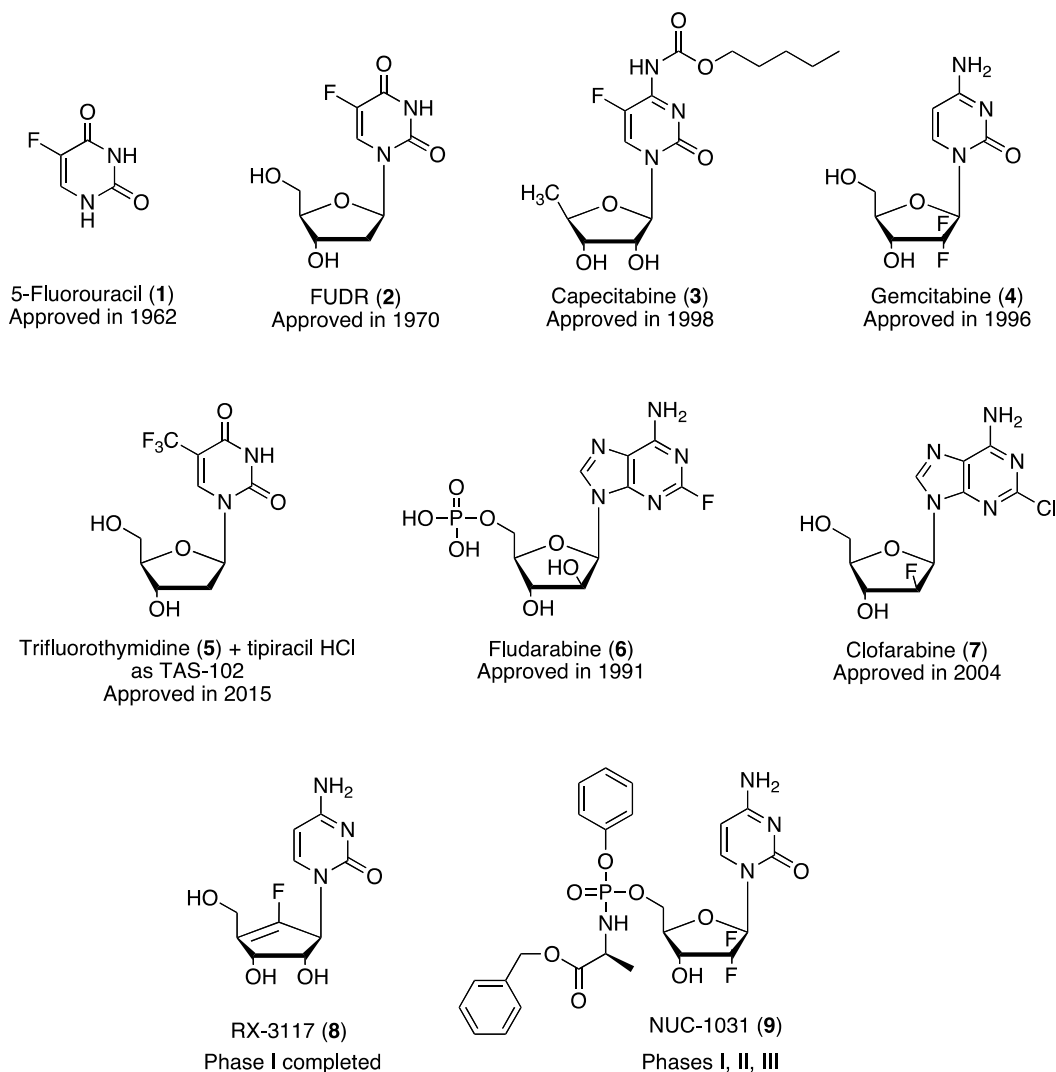
emission energy emitter. As ^{18}F is the optimal radioisotope for PET, many research efforts have been directed towards the development of efficient pathways for the synthesis of new ^{18}F -PET tracers. Currently, several ^{18}F -nucleoside analogs are being explored in clinical trials as proliferation biomarkers, as predictive tools for the patient's response to chemotherapy with nucleoside analogs, and as reporter imaging probes for the development of individually personalised medicines [3]. However, the synthesis of such NAs is challenging as the insertion of ^{18}F -fluorine into NAs should preferably be at a late synthetic stage, in a way that allows for the synthetic routes to prepare the labelled NAs and the imaging process to take place within several half-lives, in order to provide scanning data of high quality. The focus of this review will be several methods for the synthesis of fluorinated nucleoside analogs and their biological application based on the most recent patent literature published between 2014 and 2018. We will discuss an improved chemo-enzymatic method for the preparation of clofarabine, an established anticancer agent, and radio-synthesis methods towards ^{18}F -radiolabeled nucleoside analogs exemplified herein by [^{18}F]-FLT and [^{18}F]-FMAU. We will cover the most recent inventions related to improved synthetic methods for the preparation of sofosbuvir parent nucleoside 2'-deoxy-2'-fluoro-2'-C-methyluridine and sofosbuvir, and highlight patent applications disclosing procedures with novel intermediates used for the synthesis of this blockbuster anti-HCV agent. Furthermore, miscellaneous applications of fluorinated nucleosides will be exemplified by RNA interference agents (RNAi), amphiphilic glycosyl nucleosides and deoxyribocyclic dinucleotides used as modulators of gene expression, supramolecular systems suitable for decontamination of aqueous mediums, and as activators of the STING receptor.

Anticancer fluorinated nucleoside analogs

An important family of FDA-approved drugs is comprised of fluorine-containing nucleoside analogs (NAs). Similarly to other modified NAs, fluorinated nucleosides mimic natural nucleosides in terms of their cellular uptake and metabolism. Their biological activity is exhibited most often through the action of 5'-triphosphate metabolites. These active species are formed, after nucleoside transport-mediated uptake, via an intracellular step-wise phosphorylation. NAs in their active-phosphorylated forms can suppress cell growth and division by inhibition of *de novo* synthesis of DNA and RNA precursors [4] as well as by inhibition of intracellular enzymes such as human or viral polymerases, ribonucleotide reductase (RNR) [5] and thymidylate synthase (TS) [6]. Anticancer fluorinated nucleoside analogs are represented by 5-fluorouracil (5-FU, **1**), which is one of the first anticancer drugs ever approved by FDA, and it is part of the essential medicines present in WHO list (Figure 1). 5-FU, together with its 2'-deoxy ribose analog 5-fluoro-2'-deoxyuridine (FUDR, **2**) is approved and currently used to treat solid tumours, including gastric, colon, breast and ovarian carcinomas. The

main mechanism through which **1** and **2** exert their biological activity is demonstrated at the level of 5'-monophosphate form by targeting thymidine synthase (TS). The TS enzyme is trapped in a ternary complex (TS/FdUMP/mTHF), thus irreversibly losing its enzymatic activity. As a consequence, further formation of thymidine monophosphate (TMP), which represents an essential component for the processes of DNA synthesis and repair, is altered [7,8]. 5-FU can also be incorporated into RNA and thus disrupt normal RNA processing and functions [9,10]. The effectiveness of 5-FU, as well as clinically approved NAs in general, is reduced by the insurgence of resistance, which can occur according to different mechanisms: decreased cellular uptake via nucleoside-specific transporters, reduced conversion to the active 5'-monophosphate form, carried out by thymidine kinase, or less efficient inhibition of TS (as in case of **1**) [11-13], and/or up-regulation of deactivating enzymes (i.e. nucleoside deaminases, purine/pyrimidine nucleoside phosphorylase) [14,15]. In this view, a number of 5-FU prodrugs including capecitabine (**3**) have been designed in order to overcome the main resistance mechanisms and are at present either approved for clinical use or under clinical investigation [16,17]. Capecitabine is considered as an extremely important pro-drug of **1** and it is used as a front-line chemotherapy agent to treat patients with metastatic colorectal cancer and metastatic breast cancer resistant to paclitaxel and anthracycline [18].

Figure 1. FDA-approved anticancer fluorinated nucleos(t)ide analogs and selected examples of fluorinated agents in advanced stages of clinical trials.



Gemcitabine (**4**) is another fluorinated nucleoside analog used in the treatment of solid tumours including pancreatic, non-small-cell lung, ovarian, bladder and breast cancers. The active 5'-triphosphate form (dFdC-TP) of **4** is incorporated in the cellular DNA during the S-phase of the cell life cycle, causing termination of nucleic acid synthesis, thus leading to cell death. Additionally, the 5'-diphosphate form of gemcitabine (dFdC-DP) inhibits ribonucleotide reductase (RNR), an enzyme that catalyses the formation of deoxyribonucleotides from ribonucleotides essential for DNA synthesis and repair [19,20]. To improve the efficacy of **4**, and particularly to have an increased intracellular formation of its active metabolite (dFdC-TP), to yield an increased half-life in the systemic circulation, to reduce the susceptibility to inactivation by cytidine deaminase, and to increase overall bioavailability, the 4-(N) and/or 5'-sites of gemcitabine have been extensively modified, for example with the phosphoramidate approach, leading to a number of agents in clinical development such as the L-alanine based-ProTide NUC-1031 (**9**) (Figure 1), extensively reviewed in the literature [13,15,21,22]. Trifluorothymidine (TFT, **5**) is a nucleoside analogue with a CF₃ group at the 5-position of the nucleobase. TFT is phosphorylated inside the cell by thymidine kinase to the active 5'-monophosphate form (TFT-MP), which is a reversible inhibitor of the thymidylate synthase (TS)

enzyme. This action is exerted by TFT-MP through reversible binding to the enzyme active pocket [23]. TFT-MP undergoes further phosphorylation to its 5'-diphosphate (TFT-DP) and 5'-triphosphate (TFT-TP) forms. TFT-TP is incorporated into DNA growing strands of cells, causing the induction of nucleic acid fragmentation [24,25]. In 2015, a combination of tipiracil hydrochloride, a thymidine phosphorylase inhibitor used in chemotherapy, and TFT was approved as TAS-102, to treat refractory colorectal cancer. RX-3117 (**8**) is a cytosine analog of the natural nucleoside neplanocin A, characterised by the presence of a fluorinated cyclopentenyl function to replace the ribose sugar (Figure 1) [26-28]. RX-3117 is orally available and effective as an anticancer in several different human tumour xenografts. A first completed clinical trial using RX-3117 as monotherapy in patients with solid tumours has shown promising indications of safety and tolerability [29].

Among purine nucleosides, fluorinated adenosine analogs represented by fludarabine (**6**) and clofarabine (**7**) have acquired a solid position in the treatment of hematologic malignancies. Fludarabine is a clinically approved agent for the treatment of chronic lymphocytic leukemia (CLL) and acute myelogenous leukemia (AML) [30]. Fludarabine active metabolite, its 5'-triphosphate form (F-ara-ATP), is an inhibitor of DNA polymerases and also of other enzymes essential for the synthesis of cellular DNA, including the DNA primase, DNA ligase and ribonucleotide reductase [31-33]. Clofarabine is used to treat patients affected by relapsed and refractory paediatric acute lymphoblastic leukemias, and it is also under clinical investigation, in combination with other agents, to treat chronic lymphocytic leukemia, acute myelogenous leukemia and myelodysplastic syndrome [34,35]. The anticancer activity of clofarabine is achieved via three major mechanisms, and these include inhibition of ribonucleotide reductase (RNR), inhibition of DNA synthesis, and direct induction of apoptosis [36]. Furthermore, clofarabine was also reported to be involved in the inhibition of the polyadenylation process that is considered to be an essential step in cells to synthesise and maintain transcripts of mRNA [37]. Interestingly, clofarabine was recently demonstrated to act as an inhibitor of HIV-1 replication by exerting a dual action: first, this nucleoside analog reduces the dNTP substrates pool available to the virus to synthesise its DNA, and second it inhibits the DNA polymerase activity of the viral reverse transcriptase enzyme [38].

Anticancer nucleosides containing fluorine or methods for their preparation in recent patent literature

Among different synthetic strategies for the preparation of nucleoside analogs including fluorine-containing nucleosides, N-glycosylation represents the mildest and most commonly applied method [39,40]. It involves a reaction between (fluorinated) sugar and nucleobase moieties in the presence of a Lewis acid, typically SnCl_4 or TMSOTf [41]. However, depending on the starting materials used in the reaction (nucleobase and sugar), the regio- and stereoselectivity of the N-glycosylation can prove

challenging, as it often leads to complex mixtures of products. Introduction of fluorine into nucleosides can be achieved either via nucleophilic or electrophilic fluorination using fluorinating agents such as Olah's reagents, DAST and XtalFluor-E or Selectfluor [42-44]. An extensive review on synthetic methodologies for the preparation of antitumour fluorinated nucleobase 5-fluorouracil (**1**), and its nucleoside-derivatives including FUDR (**2**), capecitabine (**3**), as well as pyrimidine nucleosides fluorinated at the sugar moiety such as gemcitabine (**4**) and related non-clinical nucleosides, was recently published by Ferraboschi and colleagues [45].

A recent improved method for the preparation of anticancer agent clofarabine (**7**) has been filed in April 2014 (date of second filing April 2015) by Synbias Pharma AG, and consists of a number of steps including an enzymatic trans-glycosylation between 2-chloroadenine (**12**) and appropriate nucleosides followed by benzylation, isomerization, sulfonylation, fluorination and deprotection steps [46,47]. The key enzymatic trans-glycosylation step between 2-chloroadenine and either uridine or guanosine as nucleosides was performed with purine nucleoside phosphorylase (PNP) or a combination of uridine phosphorylase (UP) and PNP [48]. The trans-glycosylation allowed a formation of only desired β -N9-stereoisomers, thus avoiding major disadvantages of traditional procedures and providing more efficient, less laborious synthetic procedures with high yield of product formation (overall yield 32% after 6 steps). The crucial fluorination step is performed with a mixture of hydrofluoric acid and an organic Lewis base. The authors compare their invention (Figure 2) with the main and established in the art synthetic methods (a) convergent: comprising generally of a coupling reaction between the purine derivatives and a fluorinated-arabinofuranosyl moiety [49-52], and (b) divergent: consisting of the introduction of fluorine into a pre-formed nucleoside analog by chemical transformations [53]. All the above-mentioned established methods have some disadvantages associated with either i) low stability of the starting material (fluorinated sugar); ii) challenging separation of the coupling product from the number of isomers (β -N9, α -N7, β -N7) formed during the reaction. In this view, the chemo-enzymatic method for the preparation of clofarabine can be considered as more advantageous over these previously reported procedures, despite more steps are involved in the overall synthetic route.

Figure 2. General synthetic method for the synthesis of clofarabine [46].

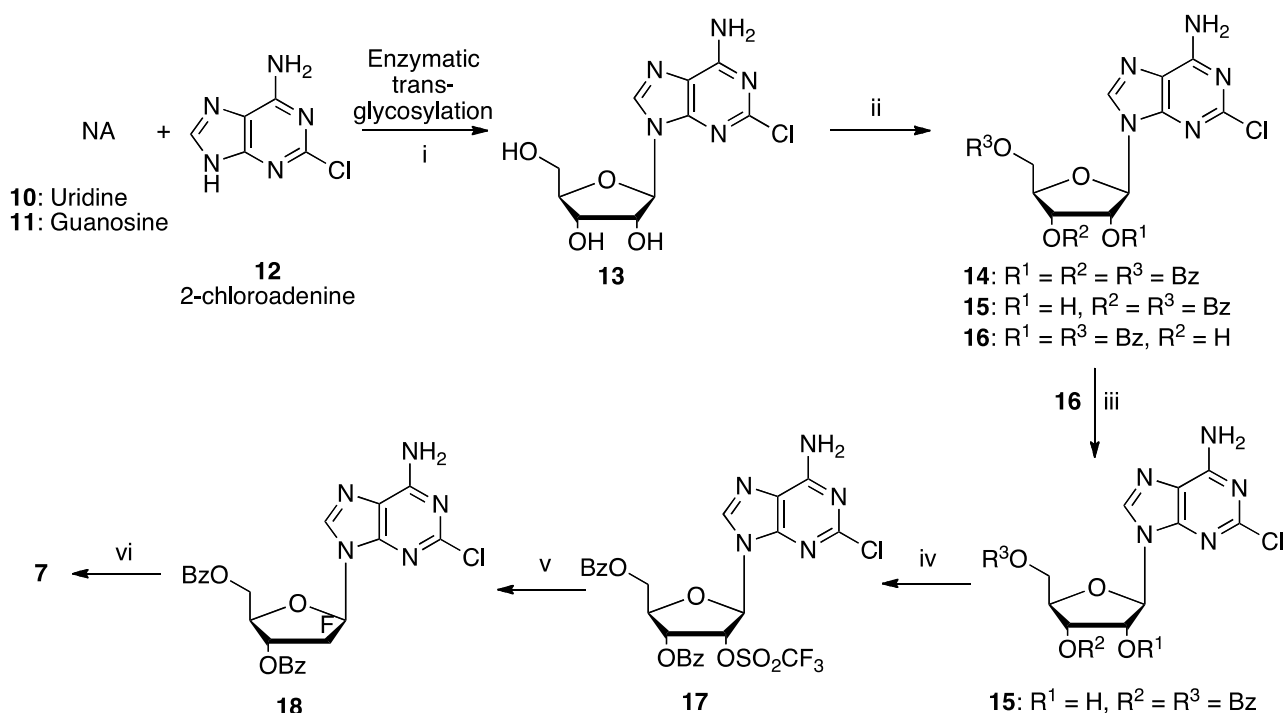


Figure 2. Reagents and conditions: (i) PNP, UP; (ii) BzCl, PyH, CH₃CN; (iii) MeOH, Δ; (iv) Tf₂O, PyH, CH₂Cl₂; (v) NEt₃·3HF, NEt(*i*Pr)₂; (vi) MeONa, MeOH.

The following two patent applications describe the preparation and the evaluation of anticancer activity *in vitro* of fluorinated pyrimidine nucleosides [54] and their silylated prodrugs [55]. The aim of the first invention patented in 2014 was to provide: a) methods for the preparation of fluorinated pyrimidine nucleoside analogs; and b) methods for inhibiting DNA methyltransferase and treating solid and hematologic cancers by these agents. The general synthetic route towards such modified nucleosides [54] consists of the typical coupling reaction between a sugar fragment (**19**) and an activated (silylated) nucleobase, namely 5-azacytosine, 2-hydroxypyrimidine and 5-(trifluoromethyl)uracil. A number of fluorinated pyrimidine analogs such as 2',2'-difluoro-5-azadeoxycytidine (**25**), 2',2'-difluorozebularine (**26**), 2',2'-difluorodeoxyribose-trifluorothymidine (**27**) were prepared as reported in Figure 3. The 2',2'-difluoro-5,6-dihydro-5-azadeoxycytidine **29** was prepared by treating the intermediate **22** with sodium borohydride in acetic acid followed by the removal of the benzoyl protecting groups from derivative **28** using a standard procedure.

Figure 3. General synthetic route towards modified nucleosides [54].

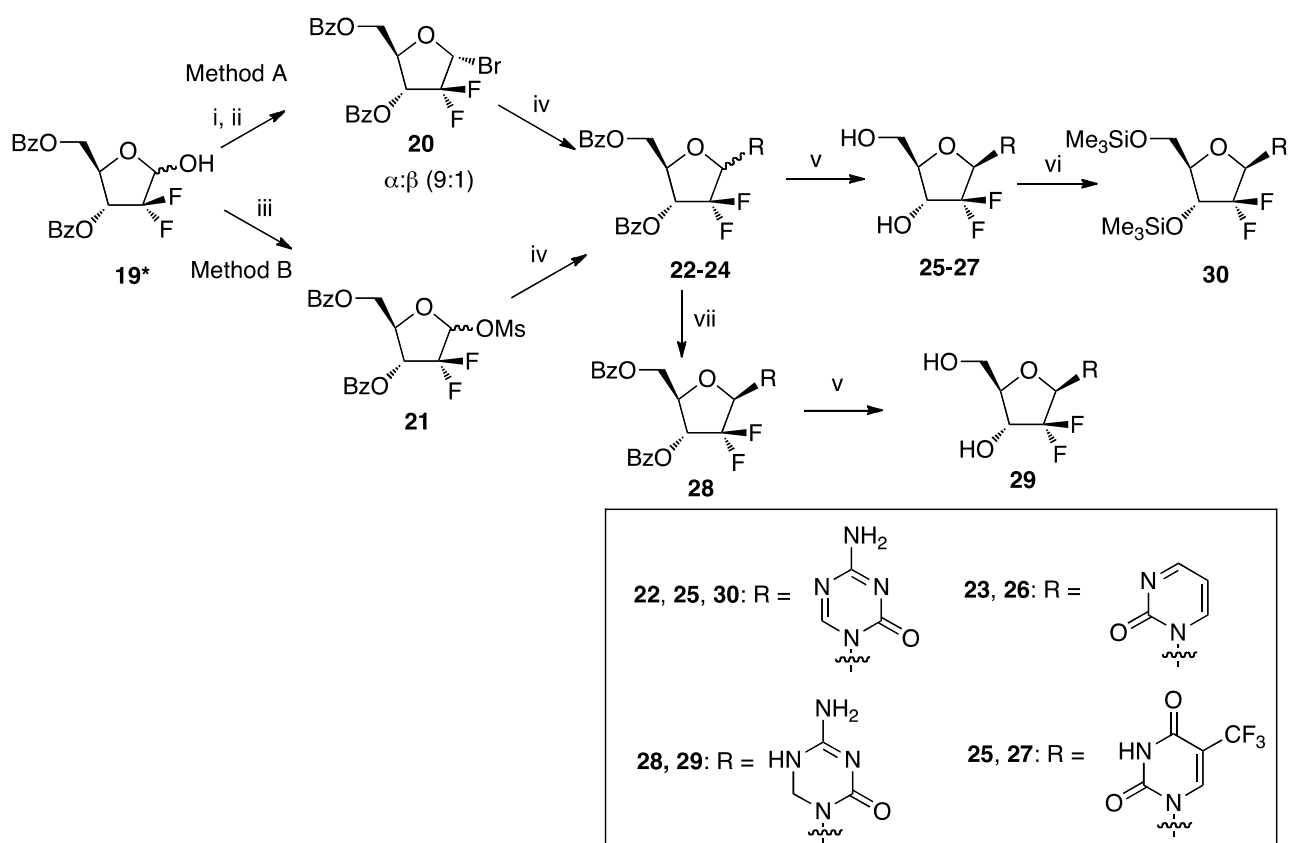


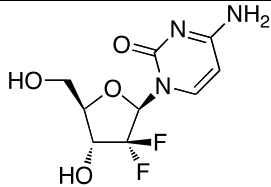
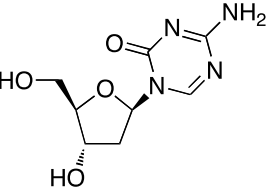
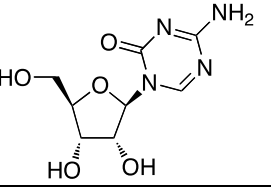
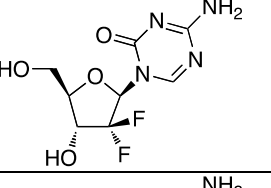
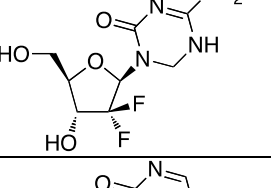
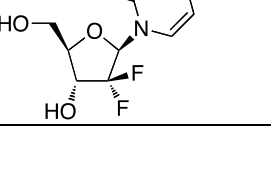
Figure 3. Reagents and conditions: (i) **19** [55], NEt_3 , toluene, $(\text{PhO})_2\text{POCl}$, 0°C to rt; (ii) HBr , acetic acid, rt; (iii) **19**, MsCl [55]; (iv) nucleobase, $(\text{NH}_4)_2\text{SO}_4$, HMDS , $(\text{CH}_3)_3\text{SiCl}$, reflux, anisole, SnCl_4 , 150°C to rt; (v) 1M NaOMe , MeOH ; (vi) TMSCl , NEt_3 , THF ; (vii) NaBH_4 , acetic acid, rt.

Among all the compounds tested, gemcitabine (**4**) revealed to be the most effective nucleoside in the inhibition of cell growth, exhibiting GI_{50} in the range of $0.01\text{--}0.72\ \mu\text{M}$ in selected cell lines, including HCT-116 (colorectal) and breast (MDA-MB-231) cell lines, respectively. The gem-fluorinated analog of 5-azacytidine, the compound NUC013 (**25**), was found to be more active in comparison with its parent nucleoside 5-azadeoxycytidine in non-small cell lung and colorectal cell lines.

The percent inhibition of DNA methyltransferase (DNMT) for selected compounds was calculated by using the DNA methyltransferase activity/inhibition assay in HT-116 cells treated with 5-azacytidine and NUC013 (**25**) at two different drug concentrations for a period of 24 h. The results demonstrated that NUC013 (**25**) is an inhibitor of DNMT at both concentrations ($1\ \mu\text{M}$ and $10\ \mu\text{M}$), with a percent inhibition higher at $1\ \mu\text{M}$ and comparable to its parent nucleoside 5-aza-C at $10\ \mu\text{M}$. The *in vitro* data (growth inhibition GI_{50}) for the fluorinated (and non-fluorinated) nucleoside analogs are reported in Table 1.

Table 1. GI_{50} in vitro data for the fluorinated and non-fluorinated nucleoside analogs [54].

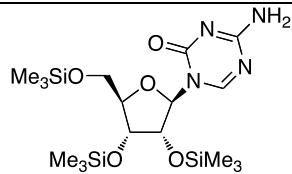
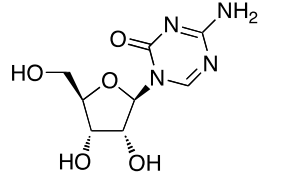
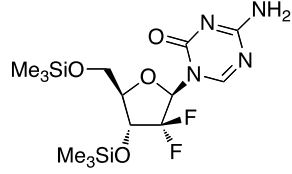
Compound	Structure	NCI-H460	MDA-MB-231	HCT-116	HL-60
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Gemcitabine (4)		0.02	0.72	0.01	0.03
5-Azadeoxycytidine (decitabine)		13.04	>31.25	>31.25	0.27
5-Azacytidine		0.43	1.64	0.62	0.44
2',2'-Difluoro-5-azadeoxycytidine (25, NUC013)		4.39	>31.25	4.54	1.51
2',2'-Difluoro-5,6-dihydro-5-azadeoxycytidine (29)		4.84	>31.25	21.40	0.03
2'-Deoxy-2',2'-difluorozebularine (26)		135.65	165.9	310.26	-

The aim of the second invention [55] was to provide a method for the preparation of prodrugs with prolonged circulatory half-life, thus providing anticancer therapeutics with potentially improved efficacy, safety, and/or pharmacokinetic profiles. In addition, formulating these hydrophobic molecules in a lipid-based nanoformulation would result in a rapid release of the active drug upon exposure to an aqueous environment at the tumour site. The introduction of a relatively non-toxic trimethylsilyl (TMS) group at the OH functional groups in the sugar moiety of selected NAs increases the hydrophobic nature of the prodrug, which is a prerequisite for further hydrophobic nanoformulation. The concept of incorporation of a bulky silyl group into 5'-OH position of nucleoside analogs was also applied in the antiviral field and led to the discovery of 5'-silylated 3'-1,2,3-triazolyl thymidine analogs as inhibitors of West Nile Virus and Dengue Virus by binding to the viral methyltransferase (MTase) [57,58]. Moreover, the silylation strategy may potentially provide other benefits like for example enhanced permeability and retention (EPR), a property by which compounds with certain size tend to accumulate at a higher concentration in tumour rather than in normal tissues [59,60]. This is particularly important in the case of nucleoside analogs unstable in

water such as 5-azacytidine, prone to fast hydrolytic cleavage of the nucleobase ring. Four fluorinated nucleosides were prepared based on the method reported in the earlier invention (Figure 3) [54]. In addition, two silylated prodrugs of 5-azacytidine and 2',2'-difluoro-5-azadeoxycytidine (**25**) were further obtained by their treatment with an excess of trimethylsilyl chloride and trimethylamine in THF as shown in Figure 3 (step vi). This is a quite straightforward method that led to the formation of compounds **31** and **30**, respectively. The latter silylated prodrug, although disclosed in the patent without *in vitro* data, was further successfully formulated in a nanoemulsion (at the concentration of 3 mg/ml) and its toxicity was tested in mice. Animals were injected with the prodrug **30** (doses up to 60 mg/kg) on three consecutive days and observed at the end of one week. No evidence of toxicity was shown as measured by weight loss up to the highest dose. The silylated prodrug (**31**) was tested in the *in vitro* cytotoxicity assay and showed comparable IC₅₀ (μM) values with its parent nucleoside 5-azacytidine (Table 2). The inventors postulated that silylated 5-aza-nucleoside analogs prodrugs may potentially demonstrate superiority *in vivo*, where a longer circulating half-life is crucial.

Table 2. In vitro cytotoxicity of NUC025 (**31**) and 5-azacytidine (IC₅₀ in μM) [55].

Compound	Structure	Cell lines		
		MDA-MB-231	HCT-116	L1210
NUC025 (31)		3.28	1.08	1.18
5-Azacytidine		1.64	0.62	1.7-2.4
2',2'-Difluoro-5-azadeoxycytidine-trimethylsilyl (30)		ND	ND	ND

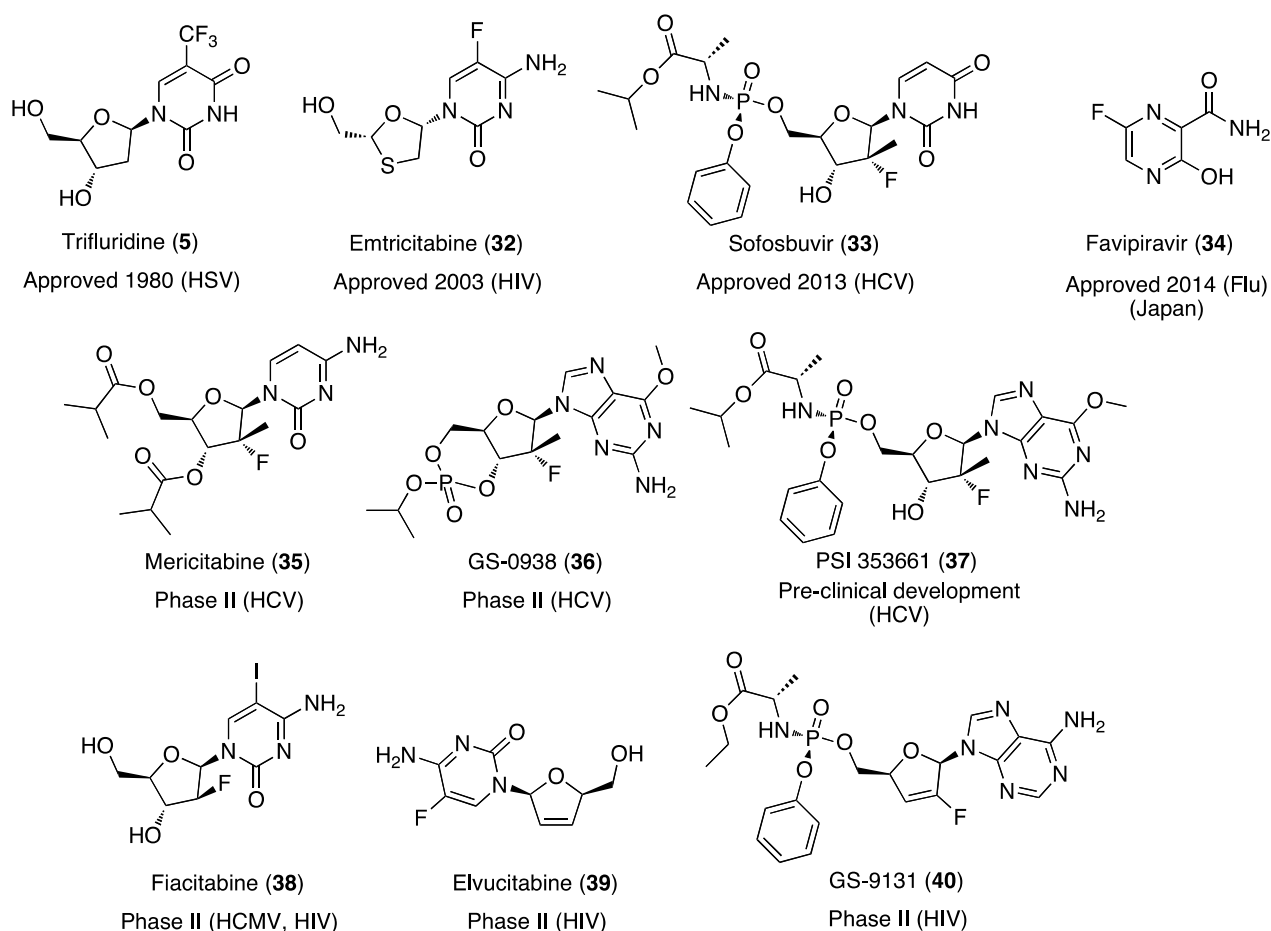
ND - no data reported

Fluorinated nucleosides in antiviral therapy

Nucleoside analogs constitute a fundamental family of compounds used as antivirals, as most therapies against extremely important human pathogens are based on the administration of nucleoside agents, including the treatment strategies for herpes simplex virus (HSV), varicella zoster virus (VZV), human cytomegalovirus (HCMV), human immunodeficiency virus (HIV) and hepatitis B virus. With some exceptions, these compounds usually exert their antiviral effect by inhibiting an

essential step in the virus life cycle, the replication of the viral genome, by targeting the viral polymerase (pol) or reverse transcriptase (RT) enzymes. The active form of these nucleosides targeting the viral nucleic acid synthesis is the 5'-triphosphate species. As in the case of anticancer nucleosides, different prodrug strategies have been developed to deliver the nucleoside monophosphate into the infected cells, as the rate-limiting step for the activation of nucleoside analogs to their triphosphate forms is the first phosphorylation into the monophosphate species. As a means to significantly improve their biological profiles and metabolic properties, fluorine is present in the structures of different antiviral nucleosides currently approved or in clinical trials. Fluorine-containing antivirals or candidate antivirals show the presence of this halogen both at the nucleobase level and at the sugar level, as highlighted in Figure 4, which summarises FDA-approved fluorinated antiviral nucleoside analogs (**5**, **32-34**) and the main antiviral candidates in clinical development belonging to this class (**35-40**).

Figure 4. Fluorinated antiviral nucleosides in the market or in advanced stages of clinical trials.



The first approved fluorinated antiviral nucleoside was trifluridine (TFT, **5**), which is an anti-herpes virus drug approved in 1980 and it is used mainly as eye-drops to treat keratitis and keratoconjunctivitis associated with herpes simplex 1 and 2 infections to the eye [61]. In combination with

the thymidine phosphorylase inhibitor tipiracil, trifluridine is also used for the treatment of metastatic colorectal cancer [22,23,62]. Another noteworthy fluorinated nucleoside is emtricitabine (FTC, **32**), a fluorinated analog of the L-nucleoside lamivudine (3TC), FDA-approved in 2003 for the treatment of HIV infection. FTC acts as a nucleoside reverse transcriptase inhibitor (NRTI) and it is part of several fixed-dose combination drugs approved to control and prevent the infection, such as Atripla, Stribild, Sustiva, Descovy and Truvada [63]. Due to its fundamental role in highly active antiretroviral therapy for HIV, FTC is included in the WHO's list of essential medicines [64].

One fluorinated nucleotide prodrug that has revolutionised antiviral therapy in the case of hepatitis C virus (HCV) is sofosbuvir (**33**), a nucleoside phosphoramidate (ProTide) whose active species potently inhibits the HCV NS5B RNA-dependent RNA polymerase [65]. Before the first direct-acting antiviral agents, HCV protease inhibitors were introduced, HCV management was non-specific for the virus and effective only in 50% of infected patients; these protease inhibitors, however, were genotype-specific and associated with severe side-effects and the insurgence of drug-resistance [66]. The approval of sofosbuvir in 2013 has completely changed the perspectives for HCV treatment: this is the first anti-HCV drug to show antiviral activity across all main genotypes of the virus, and when administered in combination with protease inhibitors and/or multifunctional NS5A enzyme inhibitors, it leads to complete clearance of the virus in most treated patients, providing the basis for all-oral, interferon-free treatments that have made possible to cure chronic hepatitis C [67].

Another fluorinated nucleobase analog in the market as antiviral is favipiravir (T-705, **34**), approved in 2014 in Japan as a measure to prevent influenza pandemics [68]. Due to its pyrazine structure, T-705 acts as a pseudo-base and, mimicking a purine analog, it is converted into the ribofuranosyl 5'-triphosphate metabolite, inhibiting the virus RNA-dependent RNA-polymerase [69]. Since its discovery, different additional mechanisms such as lethal mutagenesis have been reported to contribute for its antiviral effect [70], and these multiple ways to interfere with viral replication might be at the basis for the broad-spectrum antiviral activity of this compound, which shows an antiviral effect against different emerging and re-emerging RNA viruses such as Chikungunya virus, Zika virus and, most importantly, Ebola virus [71].

Several fluorinated antiviral candidates are also in advanced clinical trial stages. Among them mericitabine (**35**) and GS-0938 (**36**) reached Phase II clinical trials for the treatment of HCV [72]. However, further clinical development of these two agents has been terminated due to a lack of significant clinical benefits [73,74]. Both these nucleoside analogs target the viral NS5B polymerase in their active triphosphate forms, and they represent different prodrugs of the same 2'-methyl, 2'-fluoro ribose sugar present in sofosbuvir, differing for the nucleobase, cytidine in mericitabine (**35**) and protected guanine in GS-0938 (**36**), respectively. PSI-353661 (**37**), which is in pre-clinical

development for the treatment of HCV, is characterised by the same fluorinated nucleoside template of GS-0938, but shows the presence of a phosphoramidate prodrug moiety at the 5'-sugar position [75].

Other fluorinated nucleoside analogs under investigation include fiacitabine (**38**), in Phase II trials for human cytomegalovirus (HCMV) and HIV infections, and elvucitabine (**39**), in Phase II trials as HIV agent, which also displays potent activity against HBV [76]. Finally, GS-9131 (**40**) is the amidate prodrug of a nucleoside 5'-phosphonate, characterised by a 2'-fluoro-2',3'-unsaturated sugar, in Phase II clinical trials for the treatment of HIV [77].

Antiviral fluorinated nucleosides or methods for their preparation in recent patent literature

The most recent patent on antiviral fluorinated nucleosides has been filed in September 2017 by Andrei *et al.*, and it concerns the preparation of novel acyclic nucleoside phosphonates as antiviral agents to treat or prevent HBV, HIV, HCMV and VZV [78]. The authors describe the preparation of novel phosphonoamidate prodrugs of acyclic nucleoside phosphonates with a 3-fluoro-2-(phosphonomethoxy)propyl side chain, and the evaluation of their antiviral activities against the four viruses mentioned above. As depicted in Figure 5, the novel compounds exemplified include the natural thymidine, adenine, cytosine and guanosine nucleobases, along with 2-amino-6-(4-methoxythiophenol)purine, 7-deaza-guanine, 7-deaza-7-fluoroguanine and 7-deaza-7-cyano-guanine. Once the nucleobase is functionalised with the acyclic fluorinated phosphonate ester **41** and the two ester groups are cleaved to yield the corresponding free phosphonic acids, as shown in Figure 5, the free acids are then converted into the corresponding aryloxyphosphonamidates (**42-50**).

Figure 5. General synthetic route towards aryloxyphosphonamidates **42-50** [78].

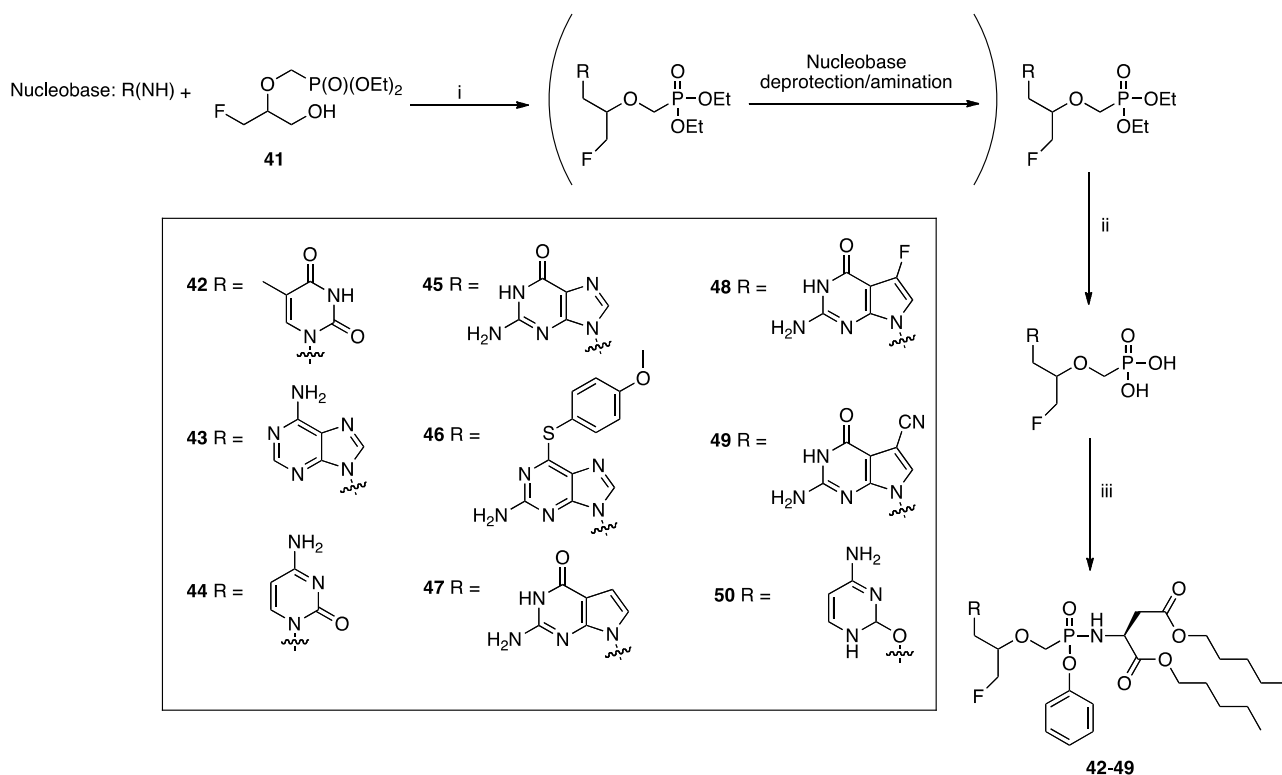
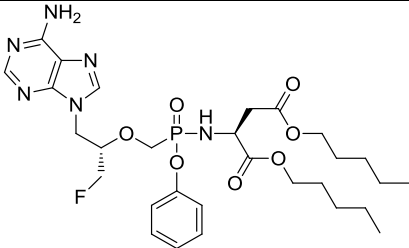
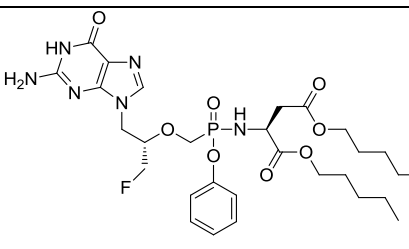
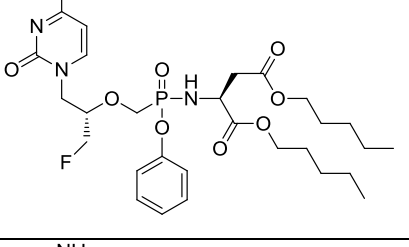
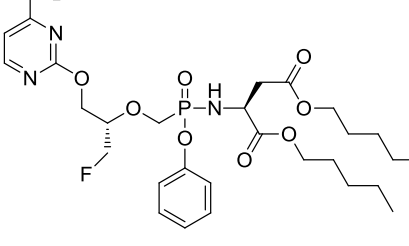
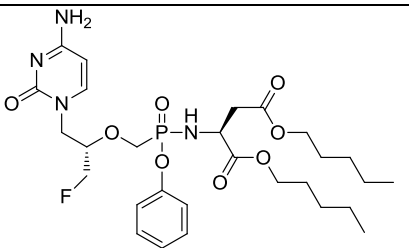
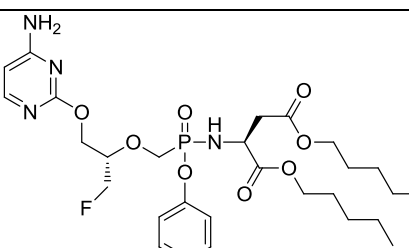
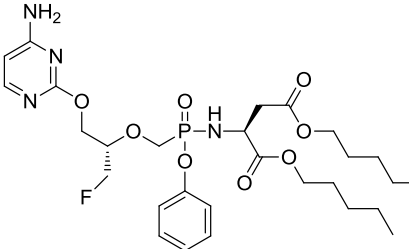


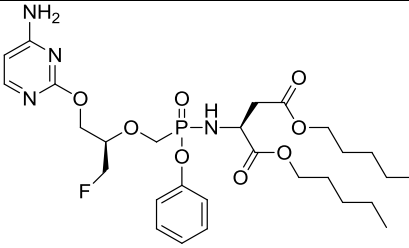
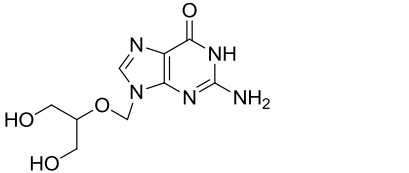
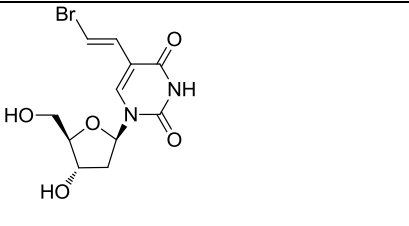
Figure 5. Reagents and conditions: (i) PPh₃, DIAD, THF, rt, 24 h; (ii) TMSBr, 2,6-lutidine, CH₃CN, rt, 12 h; (iii) L-aspartic acid amyl diester HCl salt, PhOH, 2,2'-dithiopyridine, PPh₃, NEt₃, Pyr, 60 °C, 12 h [78].

All the diastereomeric mixtures at the 3-(fluoropropan-2-yl)oxy)methyl chiral carbon have been separated, the two diastereoisomers for each couple have been evaluated against HBV, HIV, HCMV and VZV replication in cellular systems, and potent inhibitors of the viral replications have been identified among these new fluorinated analogs, which also appear to be non-toxic and show high selectivity indexes. The most significant antiviral data are reported in Table 3. In most cases, the chirality on the 3-(fluoropropan-2-yl)oxy)methyl chiral carbon is key for antiviral activity, as there is a marked difference in the antiviral potential for all S/R diastereomeric couples at this carbon.

Table 3. Antiviral and cytotoxicity data for the most active novel analogs reported by Andrei *et al.* [78].

Compound	Structure	Virus	EC ₅₀ (μM)	CC ₅₀ (μM)
3TC (Lamivudine)		HBV	0.02	>2.00
Entecavir		HBV	<0.01	>1.00

43 (S)		HBV	0.01	22.56
		HIV-1	0.01 (X4 strain NL4.3) 0.0042 (R5 strain BaL)	33.21
43 (R)		HBV	1.06	33.03
		HIV-1	0.14 (X4 strain NL4.3) 0.11 (R5 strain BaL)	37.61
45 (S)		HBV	0.13	>100
		HIV-1	0.16 (X4 strain NL4.3) 0.22 (R5 strain BaL)	>100
45 (R)		HBV	0.02	>100
		HIV-1	0.073 (X4 strain NL4.3) 0.06 (R5 strain BaL)	>100
44 (S)		HCMV	0.76±0.10 (AD-169 strain) 2.02 (Davis strain)	74.7±9
		VZV	3.15±2.61 (Oka strain) 1.32±0.46 (strain 07-1)	
44 (R)		HCMV	>32.6 (AD-169 strain) >32.6 (Davis strain)	ND
		VZV	>32.6 (Oka strain) >32.6 (strain 07-1)	
50 (S)		HCMV	17.6±4.3 (AD-169 strain) 16.2±2.3 (Davis strain)	46.9±2.20
		VZV	3.96±1.147 (Oka strain) 1.32±1.50 (strain 07-1)	

50 (R)		HCMV VZV	0.029±0.0046 (AD-169 strain) 0.033±0.0081 (Davis strain) 0.034±0.026 (Oka strain) 0.013±0.0096 (strain 07-1)	>163
Ganciclovir		HCMV VZV	8.70±0.66 (AD-169 strain) 16.25±22.5 (Davis strain) ND	>391
Brivudin		HCMV VZV	ND 0.055±0.019 (Oka strain) 5.21±3.97 (strain 07-1)	>300

Two recent patents on antiviral fluorinated nucleosides are focussed on the preparation of the blockbuster anti-HCV drug sofosbuvir (**33**). The first, filed by Sandoz in 2016 [79], protects an improved fluorination method for the preparation of sofosbuvir-based nucleoside starting from 2'-C-methyl-arabino-uridine **51**, which is prepared according to a reported procedure [80]. As shown in Figure 6, different protecting groups are explored in this patent for the protection of the 3'- and 5'-positions of the starting nucleoside, and on these protected molecules an alternative deoxyfluorinating agent is used to achieve deoxyfluorination in position 2': XTalFluor E, (diethylamino)difluorosulfonium tetrafluoroborate, is used as the fluoride source. This tetrafluoroborate salt replaces DAST, used in the previous art, and it is associated with a significantly improved fluorination yield on the nucleoside substrate when applied together with the alternative protecting group, CCl₃CO, introduced for the first time in the invention, as summarised in Table 4.

Figure 6. Synthetic method for the preparation of sofosbuvir-based nucleoside **55** and sofosbuvir (**33**) [79].

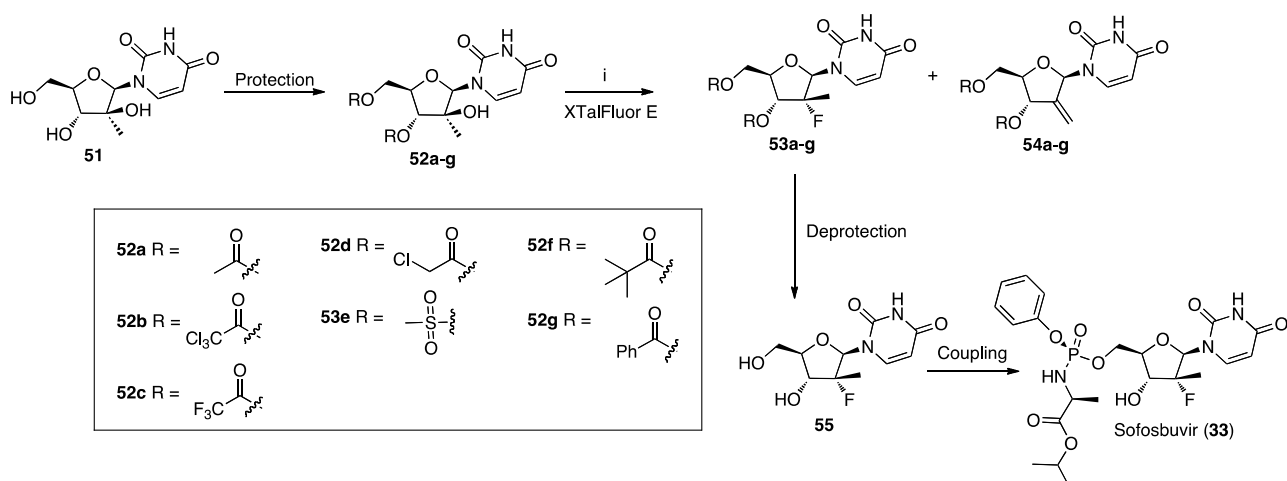


Figure 6. Reagents and conditions: (i) NEt_3 , $\text{TEA} \cdot 3\text{HF}$, DCM, rt, 4 h [79].

Table 4. Yields of desired products **53** and undesired unsaturated by-products **54** in the presence of different protecting groups for the nucleoside substrates and different deoxyfluorinated agents, DAST, as per the previous art, and XTalFluor E, as presented in the invention. [79]

Protecting group (R)	Deoxyfluorinating agent	Yield % of target compound 53	Yield % of unsaturated 54
CF_3CO	XTalFluor E	42 % (deprotected)	37% (deprotected)
CH_3CO	XTalFluor E	<26% (protected, not isolated)	N.D.
CH_3CO	XTalFluor E	<26% (protected, not isolated)	N.D.
ClCH_2CO	XTalFluor E	47% (protected)	24% (protected)
CCl_3CO	XTalFluor E	46% (after deprotection) 58% (no deprotection)	N.D.
CCl_3CO	DAST	<20% (protected, not isolated)	N.D.
CH_3SO_2	XTalFluor E	26% (protected)	63% (protected)
<i>t</i> -Bu-CO	XTalFluor E	18% (protected)	7% (protected)
Ph-CO	XTalFluor E	12% (protected)	2% (protected)
Ph-CO	DAST	20% (protected)	42% (protected)

A second Chinese patent filed in 2017 describes an alternative method for the preparation of sofosbuvir starting from β -D-ribose and using, according to the inventors, cheaper raw materials and synthetic methods in comparison with the previous art [81]. The proposed economic and easy to scale-

up strategy is composed of eight synthetic steps from β -D-ribose, as shown in Figure 7.

Figure 7. Economic and easy to scale-up strategy for the preparation of sofosbuvir (**33**) [81].

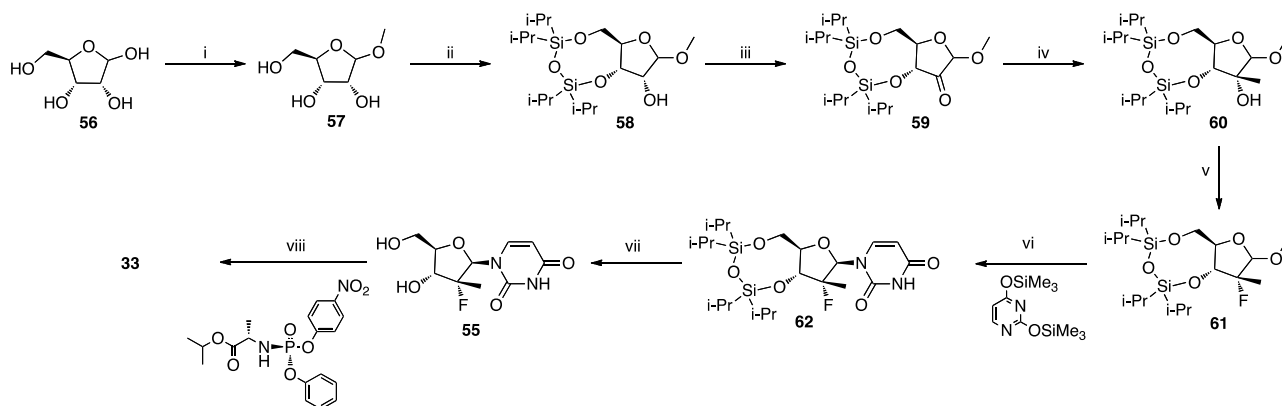


Figure 7. Reagents and conditions: i) Acetyl chloride, MeOH, 0 °C to rt, 24 h; ii) TIPDSCl, DCM, Pyr, 0 °C to rt, 3 h; iii) PhMe, EtOAc, TEMPO, aq. NaHCO₃, NaOCl, 0 °C, 2 h; iv) MeMgCl, Et₂O, 0-10 °C, 3 h; v) DAST, DCM, THF, rt, 3 h; vi) SnCl₄, MeCN, 0 °C to rt, 4 h; vii) DMF, glacial AcOH, NMe₄F, rt, 2 h (95%); viii) *tert*-BuMgCl, THF, rt, 48 h (68%) [78].

Briefly, in this synthetic strategy, the 1'-hydroxyl function of β -D-ribose is converted to methoxy using acetyl chloride in methanol (Figure 7, step i). This substrate (**57**) is then protected with a tetraisopropylidisiloxane group at the 3'- and 5'-positions, and the free hydroxyl group in 2' is then oxidised to ketone using sodium hypochlorite and TEMPO (Figure 7, steps ii and iii). The 2'-keto intermediate (**59**) is then treated with a Grignard reagent to afford the methylation at the 2'-position, and this methylated sugar (**60**) is then fluorinated in 2' using DAST (Figure 7, steps iv and v). The 2'-methyl-2'-fluoro protected sugar (**61**) is then coupled with silylated uridine in anhydrous acetonitrile using tin (IV) chloride as Lewis acid. The silylated nucleoside (**62**) is deprotected using acetic acid and tetramethylammonium fluoride, and the free key nucleoside **55** is finally reacted with (2S)-2-(((4-ylphenoxy)phenoxyphosphoryl)propionic acid isopropyl ester in tetrahydrofuran using the strong Grignard reagent *tert*-butylmagnesium chloride as base to give sofosbuvir (**33**). Even if the authors claim that this represents an improved method for the preparation of sofosbuvir, no comparison with the previous art is possible, based on the data provided as examples in the document: reaction yields are given only for the two last steps of the synthetic scheme, no indication is present on the anomeric selectivity of the methylation and fluorination steps, and no information is given on how the sugar with the desired configuration at the 2'-position is isolated, and what is the yield of the desired sugar configuration over the other.

Over the last five years a number of patent applications enclosing procedures for the diastereoselective preparation of sofosbuvir with high purity level and improved yield, physical and

chemical properties were reported, as summarized in Table 5 [82-85]. In general, methods disclosed in these patents are based on novel synthetic schemes and use of novel intermediates and phosphoramidating agents employed in the preparation of sofosbuvir.

Table 5. Methods for the preparation of sofosbuvir-related patent applications.

Patent application number and title	Context of the disclosure	Ref
WO 2017/009746 “An improved process for the preparation of sofosbuvir”	Use of a novel reagent (magnesium di- <i>tert</i> butoxide instead the standard <i>t</i> BuMgCl) as a base for the coupling reaction (Figure 7, step viii) Use of novel type of oxyimino-phosphoramidating reagents for the coupling reaction (Figure 7, step viii)	[82]
WO 2017/093973 “Process for the preparation of pure sofosbuvir”	Use of “bromo-sofosbuvir” as an intermediate compound to obtain sofosbuvir	[83]
WO 2016/008461 “A new form of sofosbuvir and a method for its preparation”	New polymorphic form of sofosbuvir, its preparation and characterisation	[84]
US 2016/0318966 “Process for the preparation of sofosbuvir”	Use of protected nucleoside intermediate and a single diastereomer phosphoramidating reagent [86] for the coupling reaction (Figure 7, step viii)	[85]

The term “bromo-sofosbuvir” refers in the patent [83] to (*S*)-isopropyl 2-((*S*)-(((2*R*,3*R*,4*R*,5*R*)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-2-yl)-(4-bromo-phenoxy)phosphorylamino)propanoate.

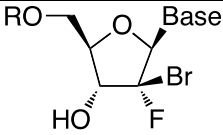
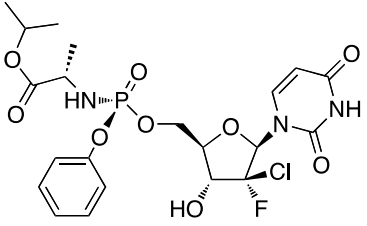
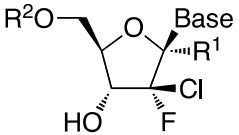
Antiviral nucleotide prodrugs

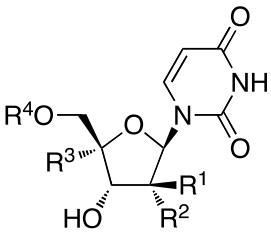
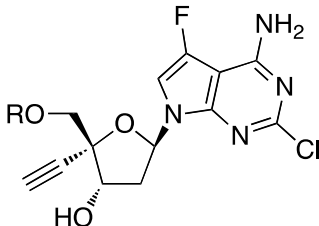
The concept of phosph(on)ates prodrugs and aryloxyphosphoramidates (ProTides) in particular, as a

technique to confer better efficacy profiles to nucleoside analogs, was extensively investigated for the last three decades [22,44,87]. This technology, which has been extensively applied to several different modified nucleoside with both antiviral and anticancer properties, continues to be of great scientific interest. A considerable amount of patent inventions directed to methods of synthesis and use of phosph(on)ates prodrugs was published in the literature [88-93]. Due to a limited scope of the present review and its main focus on fluorinated nucleosides, in this section only selected and most recent patent applications are briefly highlighted with general examples of fluorinated-nucleos(t)ide analogs disclosed, as reported in Table 6.

Table 6. Selected patent applications disclosing methods for the preparation of nucleoside and nucleotide analogs thereof [88-93].

Patent number and title	Context of the disclosure General formula of compound(s) disclosed	Ref
<p>US 2015/0366888 A1</p> <p>“Substituted nucleosides, nucleotides and analogs thereof”</p>	<p>Synthesis of fluorine-containing nucleosides and their nucleotide analogs and methods for the treatment of diseases and/or conditions of viral infection caused by <i>Coronaviridae</i> virus, a <i>Togaviridae</i> virus, a <i>Hepeviridae</i> virus.</p> <div data-bbox="890 1182 1129 1317" style="text-align: center;"> <p>General formula 1</p> </div> <p>Base: pyrimidine, modified purine $R^1 = \text{H, F, CH}_3, \text{C}\equiv\text{CH}$ $R^2 = \text{Cl, F, OH, NH}_2, \text{N}_3$ $R^3 = \text{F, CH}_2\text{N}_3, \text{CH}_2\text{F, CH}_2\text{Cl, CH}=\text{CH}_2, \text{C}\equiv\text{CH}$ $R^4 = \text{H, ProTide-type masked monophosphate}$</p>	[88]
<p>WO 2016/178876 A9</p> <p>“Nucleoside analogs for treatment of the Flaviviridae family of viruses and cancer”</p>	<p>Synthetic methods for the preparation of nucleosides and their nucleotide analogs. Methods for treating and preventing <i>Flaviviridae</i> family of viruses, RSV, HEV, and influenza infection and cancer in human subjects or other animal hosts.</p>	[89]

	 <p>General formula 2</p> <p>Base: pyrimidine, modified purine R = H, ProTide-type masked monophosphate</p>	
<p>WO 2016/140615 A1</p> <p>“Nucleotide derivatives which are HCV inhibitors for use in the treatment of hepatitis C”</p>	<p>Synthetic methods for the preparation of nucleoside (1-((2R,3S,4R,5R)-3-chloro-3-fluoro-4-hydroxy-5-(hydroxymethyl)-tetrahydrofuran-2-yl)pyrimidine-2,4(1H,3H)-dione and its phosphoramidate prodrug represented by formula 3. Use of the prodrug (formula 3) in the treatment or prophylaxis of hepatitis C, including HCV genotype 3 virus infection in a combination therapy.</p>  <p>Phosphoramidate prodrug (Formula 3)</p>	[90]
<p>US 2017/0037078 A1</p> <p>“2'-Disubstituted nucleoside analogs for treatment of the Flaviviridae family of viruses and cancer”</p>	<p>Synthetic methods for the formation of 2'-disubstituted nucleoside analogs and nucleotide prodrugs and their use to treat and prevent <i>Flaviviridae</i> infections, RSV and influenza infection and cancer.</p>  <p>General formula 4</p> <p>Base: pyrimidine, modified purine R¹ = H, CN R² = H, ProTide-type masked monophosphate</p>	[91]
<p>US 2017/0057981 A1</p> <p>“Anti-viral compounds”</p>	<p>Processes for the preparation of nucleoside and nucleotide analogs and their use for the</p>	[92]

	<p>treatment of HCV infection.</p>  <p>General formula 5</p> <p>R¹ = F, Cl, Br, CH₃, CHF₂, C=CH₂ R² = F, Cl, Br, CH₃... R³ = OCH₃, CN, N₃... R⁴ = H, ProTide-type masked monophosphate</p>	
<p>WO 2017/053216 A2</p> <p>“4'-Substituted nucleoside reverse transcriptase inhibitors and preparations thereof”</p>	<p>Methods for the preparation of fluorinated-nucleosides and their nucleotide analogs as inhibitors of HIV reverse transcriptase.</p>  <p>General formula 6</p> <p>R = H, ProTide-type masked phosphate</p>	[93]

Recent patent literature on fluorinated nucleosides with miscellaneous uses, or methods for their preparation

As discussed above, several ¹⁸F-fluorinated nucleosides are under clinical investigation as probes for positron emission tomography (PET), in order to be used as biomarkers for cell proliferation in oncology, as imaging tracers for personalised medicine formulations, and as tools to predict the patient's response to nucleoside-based chemotherapy [94]. As the ¹⁸F-fluorine needs to be incorporated at a late stage in the synthesis of these probes, in order for the synthesis and the imaging steps to be performed in the timeframe of multiple half-lives, thus resulting in scanning data of high quality, the chemistry for its introduction, usually based on aliphatic nucleophilic fluorinations on the protected nucleoside, is renowned as challenging, time consuming, labour intensive, highly variable and unreliable. In order to introduce the labelled fluorine late in the synthesis, preparation of activated fluoride (moisture-free) is required: traces amounts of ¹⁸F-fluoride are sequestered into anion exchange columns, then eluted using salts dissolved in water, and finally dried from the excess water

in order to afford an activated mixture of ^{18}F -fluoride, salt (usually K_2CO_3) and a stabilising agent such as Kryptofix K_{222} . The dried mixture is then used for the labelling of the probe. This drying step is usually time-consuming, it requires elevated temperatures, and it can lead to under-drying or over-drying, which both affect negatively the labelling step. In 2015, a patent was granted on the preparation of ^{18}F -labelled tracers in hydrous organic solvent mixtures that, containing between 0.1% and 5.0% of water, do not require drying of the activated ^{18}F -fluoride mixture, thus speeding-up the process of labelling and increasing the yields of radiolabelled probes, as the activated ^{18}F -fluoride organic solution can be used directly for the radiolabelling step [95]. The patent exemplifies, among others, the preparation of ^{18}F -FLT, the first ^{18}F -radiolabelled approved for human use as tracer for tumour proliferation [96-99], as shown in Figure 8: the ^{18}F -labelling step occurs in high yield from the protected precursor Boc-Boc-Nos, with a precisely controlled amount of water and no drying step required.

Figure 8. Synthetic method to prepare ^{18}F -FLT [95].

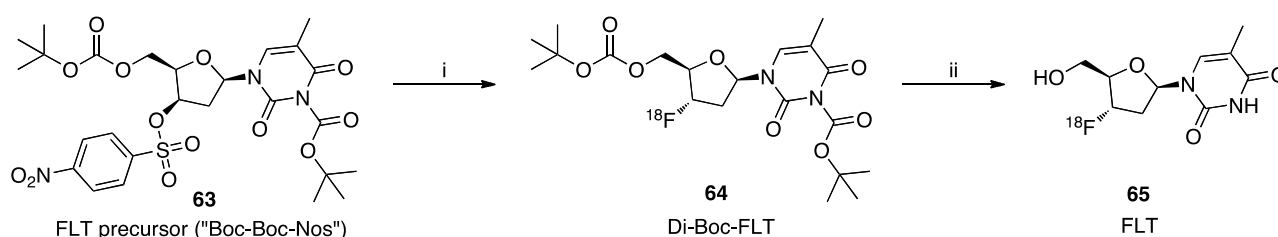


Figure 8. Reagents and conditions: i) K_2CO_3 , Kryptofix-[222], ^{18}F -Fluoride, MeCN, 135°C , 180 sec; ii) 1M HCl, 105°C , 600 sec. [95].

As reported in the patent, the first reaction step is monitored via radio-TLC, which indicates a $>90\%$ conversion of the ^{18}F -fluoride into ^{18}F -Di-Boc-FLT (**64**). Subsequent Boc-deprotection in aqueous HCl yielded the desired FLT (**65**) with a $>96\%$ purity.

Another worth-mentioning patent is focussed on the radio-synthesis of fluorinated pyrimidine nucleosides (filed in 2012 by Li *et al.*) [100], represented by ^{18}F -FMAU (**69**), an established PET probe used to monitor cellular proliferation [101,102]. The inventors reported an improved method of one-pot procedure to synthesize ^{18}F -FMAU. Furthermore, the claimed method can also be incorporated into a fully automated cGMP-compliant radio-synthesis module to produce PET probes by simplified reaction conditions in the presence of Friedel-Crafts catalysts. These ^{18}F -labelled thymidine or cytidine analogs can also be used as PET tracers for certain medical conditions including autoimmunity inflammation and bone marrow transplant [103]. The authors discuss various reaction conditions (solvents, reaction temperature, the use of different Friedel-Crafts catalysts: AlCl_3 , SnCl_4 , ZnCl_2 and TMSOTf), and their influence on the conjugation (Figure 9, step ii), yield and ratio of α/β

anomer formation for the compound **68** (Figure 9). TMSOTf was claimed as the most efficient catalyst acting also as a co-solvent, significantly improving a solubility of the silylated-base, the yield of the coupling step, and increasing the formation of the desired β -anomer. In addition, alternative methods/platforms that are suitable for the synthesis of radiopharmaceuticals such as microwave-assisted conjugation and microfluidic devices are mentioned in the patent, as they can be also used in the preparation of ^{18}F -radiolabelled nucleoside analogues exemplified in the patent with the potential to increase radio-synthesis output.

Figure 9. Synthetic method for the preparation of ^{18}F -FMAU [100].

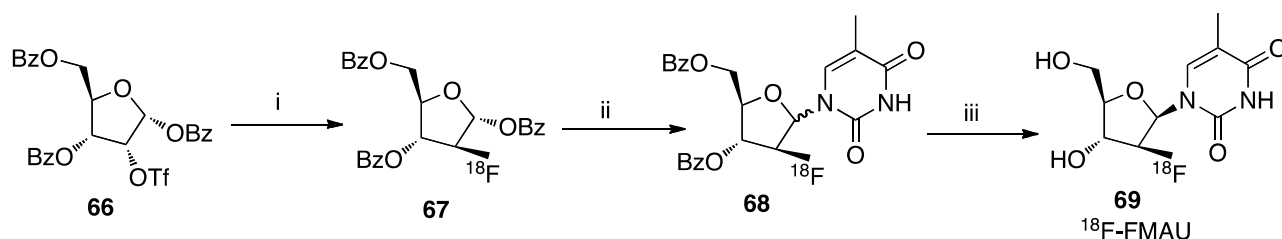
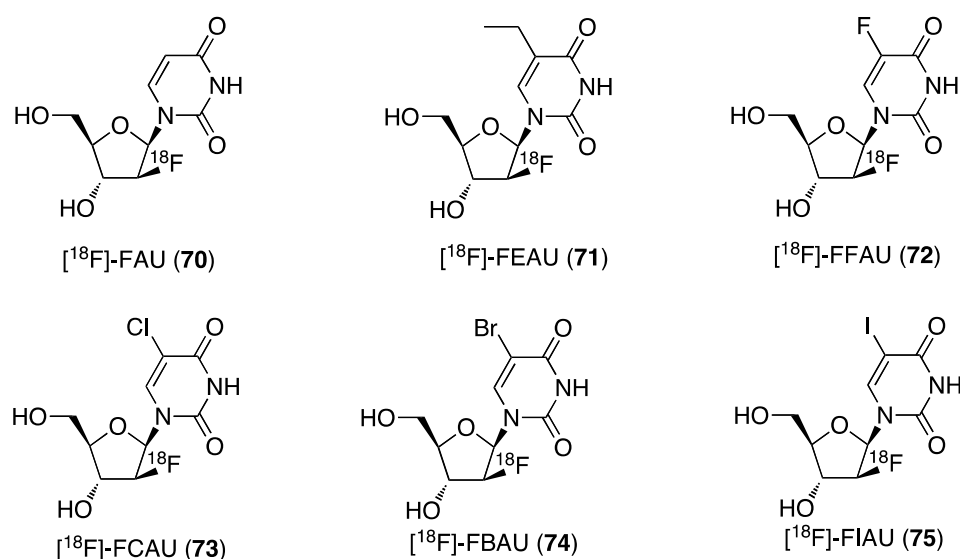


Figure 9. Reagents and conditions: (i) ^{18}F -NBu₄F, MeCN, 80 °C, 30 min. (ii) TMSOTf, HMDS (iii) NaOMe, MeOH then HPLC purification [100].

Examples of other [^{18}F]-labelled 5-substituted thymidine analogs which synthesis can be carried out with a full one-pot automation are depicted in Figure 10.

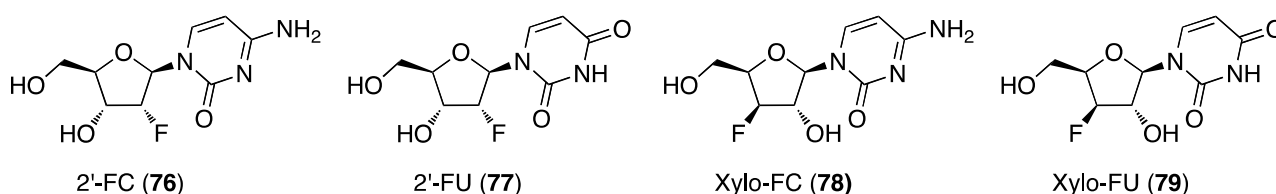
Figure 10. Examples of ^{18}F -labelled 5-substituted thymidine analogs **70-75** [100].



The recent patent focusing on the preparation of modified RNAi agents containing 2'-fluoro or 2'-methoxy-xylose analogs of natural nucleosides, and their use as modulators of gene expression, was

filed in 2014 by [104]. RNA interference (RNAi) is the effect of the administration of double-stranded RNA (dsRNA), which can block gene expression: short dsRNA are responsible for gene-specific post-transcriptional silencing in several organisms, and the reduced expression of selected target genes is correlated with several effects, such as increased host immune response against pathogens, increased expression of genes implicated in cell cycle arrest, increased expression of tumour suppressor genes. As a chemical modification to dsRNAs to improve their drug-like properties, this patent protects the preparation of double-stranded RNAi agents with 12 to 30 nucleotides in the sense strand and antisense strand, which contain at least one xylo-modified or 2'-modified nucleoside with a fluoro or a methoxy group. The fluorinated nucleosides included in the invention as building blocks for modified dsRNAs are shown in Figure 11.

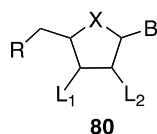
Figure 11. Modified fluorinated nucleosides inserted by Rajeev *et al.* in the sequences of dsRNAs [104].



Following a discussion of methods to prepare the target dsRNAs with the described modifications, the inventors also disclosed the improved stability of such structures towards snake venom phosphodiesterase and their activity against the blood coagulation factor VII in mice: the novel dsRNAs display IC₅₀ values between 0.0074 nM and 0.1822 nM.

In 2013, Barthelemy *et al.* have protected the use of fluorinated amphiphilic glycosyl nucleosides (GNFs) as supramolecular systems comprising low molecular weight molecules for decontaminating aqueous mediums from nanoparticles and microparticles, with an exemplified focus on gold nanoparticles (auNPs), which is an important contaminant in the cosmetic industry [105]. This invention describes the use of supramolecular systems for subtracting particles from a liquid medium, mainly water, and these supramolecular systems are made of at least one low-molecular weight molecule together with organic polymers such as collagens, polysaccharides or proteoglycans. The patent protects several fluorinated and perfluorinated molecules as depicted in Figure 12, but the only one exemplified is the compound **83**, whose synthesis had been previously published in Tetrahedron Letters in 2010 [106].

Figure 12. Fluorinated amphiphilic glycosil nucleosides considered by Barthelemy *et al.* for the hydrogel-based decontamination of aqueous media [105].

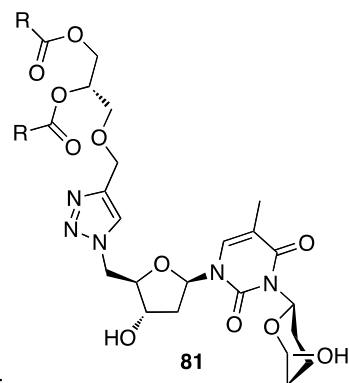


with:

X = O, S or CH₂

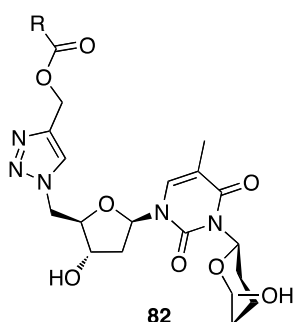
B = purine or pyrimidine base, non-natural monocyclic or bicyclic (substituted) base

L₁, L₂ = OH, H, heteroaryl, chemical spacer linked to a fluorinated or prefluorinated hydrocarbon-based chain



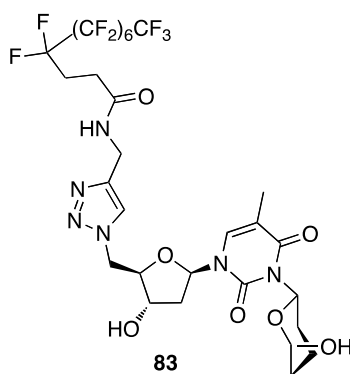
with:

R = hydrocarbon chain or fluorinated hydrocarbon chain



with:

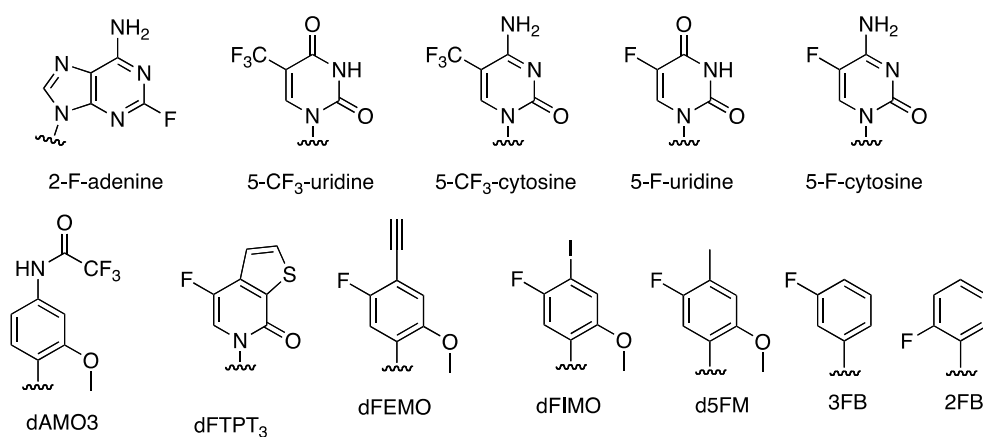
R = hydrocarbon chain or fluorinated hydrocarbon chain



In 2016, Ptacin *et al.* have protected the preparation of oligonucleotides with an expanded genetic alphabet, including several unnatural bases, unnatural sugars and also modified linkers between nucleosides [107]. Different of the unnatural modifications protected in this application contain fluorinated groups, and they are summarised in Figure 13.

Figure 13. Fluorinated unnatural bases and sugars protected by Ptacin *et al.* as building blocks for the preparation of mutant tRNAs and mRNAs [107].

Unnatural fluorinated bases



Unnatural fluorinated sugars

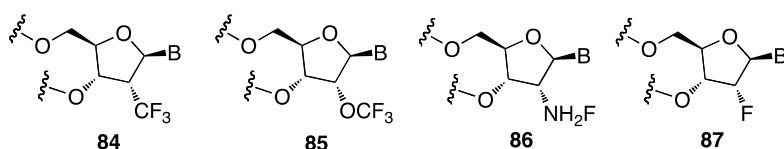


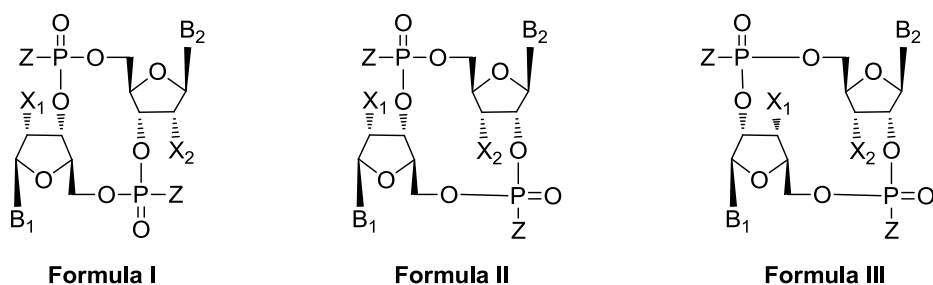
Figure 13. Fluorinated unnatural bases and sugars protected by Ptacin *et al.* as building blocks for the preparation of mutant tRNAs and mRNAs [107].

These unnatural bases and sugars are claimed to be used in the preparation of mutant tRNAs and mRNAs, according to a series of several mutant codon (tRNA) and anticodon (mRNA) sequences described in the application. These mutant tRNAs and mRNAs are then used to synthesise unnatural amino acids, which are also exemplified in the patent application. The claimed therapeutic uses of the protected method are the generation of probes, unnatural polypeptides, unnatural macrocycles, site-specific antibody-drug conjugates, bi-specific antibodies, nucleic acid catalysts, biosensors, kill switch and gene delivery. Even if all these applications are claimed and the preparation of the mutant tRNAs and mRNAs is described, no example is given on any practical use, and efficacy, of the protected unnatural nucleic acids and amino acids.

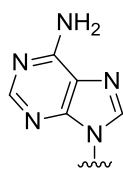
One final patent application filed in 2016 [108] describes the preparation of novel fluorinated deoxyribo-cyclic dinucleotides (CDNs) and evidences their activity as activators of the receptor STING (stimulator of interferon genes) [109,110]. By activating this receptor, the novel compounds are demonstrated to induce the production of Type I interferons and pro-inflammatory cytokines, which represents a very promising approach for immunotherapy, to enhance the immune system's responses of a human or animal in the case of autoimmune disorders, immune system deficiencies, bacterial or viral infections, other infectious diseases, or cancer. The novel fluorinated compounds protected in this invention, whose structures are summarised in Figure 14, show improved biological and pharmacokinetic properties in comparison with their non-fluorinated analogs, as demonstrated in

the patent.

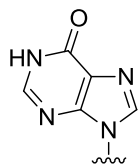
Figure 14. Summary of the structures protected by Vernejoul *et al.* as STING agonists [108].



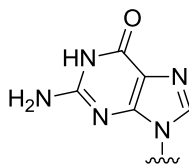
With B_1 and B_2 chosen from:



Adenine



Hypoxanthine



Guanine

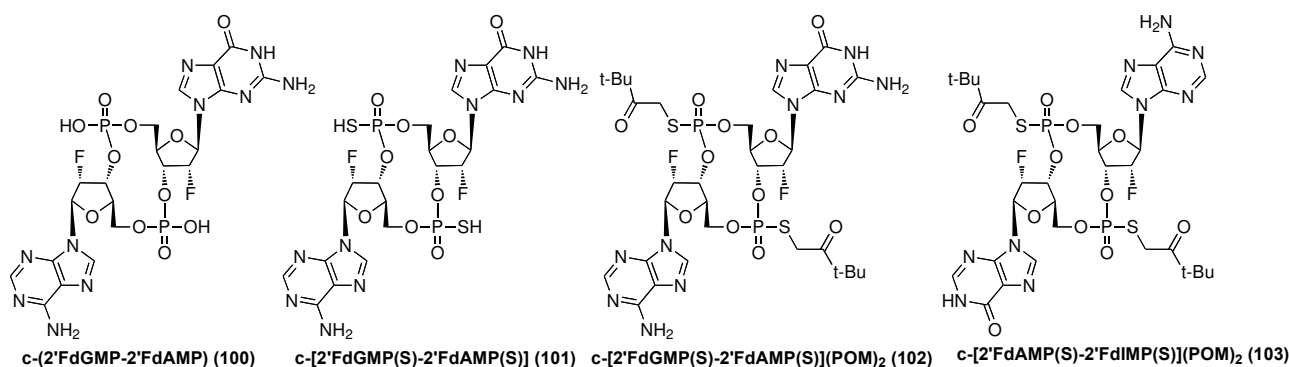
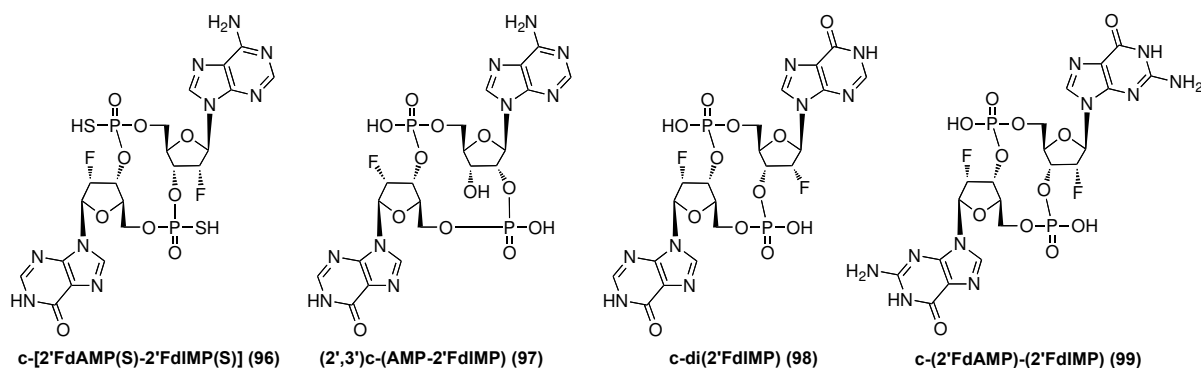
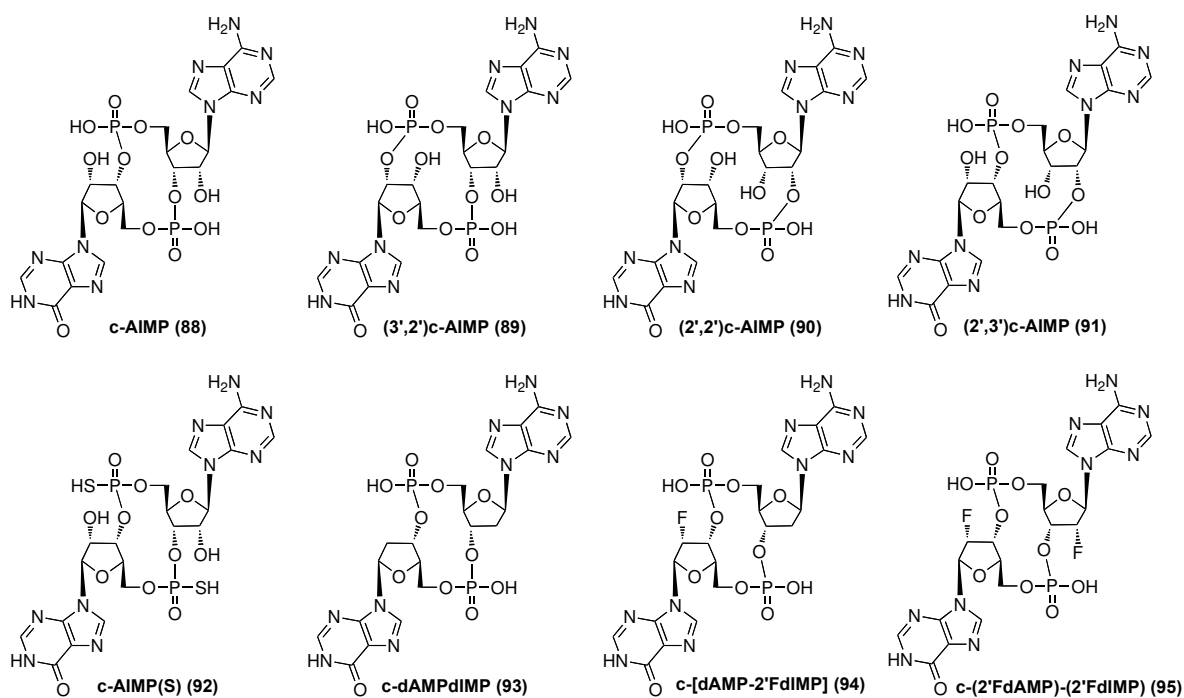
X_1 and X_2 can be F, H or OH

Z can be OH, SH, OR or SR, with R= alkyl ester, carbonate, carbamate or amide

(phosphate prodrugs)

The patent also describes in detail the chemical preparation of the novel immunotherapy agents exemplified. A summary of the exemplified structures, for which extensive biological data are also reported in the application, is given in Figure 15.

Figure 15. Compounds exemplified by Vernejoul *et al.* as STING agonists [108].



Cytokine induction in treated cell cultures has been determined for the exemplified new compounds using different cell lines, and they were confirmed to induce Type I interferons production in wild-type cells but not in STING knockout cells, confirming that they act through the activation of STING. The *in vitro* assays also confirm that the fluorinated analogs prepared are more active than the corresponding reference compounds. Cytokine induction is also evaluated *in vivo* in mice models, and the ability of the new CDNs to induce Type I interferons is confirmed. This activity is also determined *in vitro* in whole human blood from healthy donors, and also in this case their ability to

induce different cytokines is confirmed, with the fluorinated analogs showing an improved activity in comparison with their non-fluorinated counterparts. Finally, the fluorinated deoxyribo-CDNs are shown to be more resistant to enzymatic degradation by snake-venom phosphodiesterase (SVPD) and nuclease P1 (NP1) than the non-fluorinated analogs.

Future perspective

Nucleoside analogs, commonly known as antimetabolites, constitute a fundamental class of chemotherapeutic agents widely used in the clinic for many decades. Among them, fluorine-modified nucleosides are a very important class of anticancer and antiviral drugs. In this review we presented fluorine-containing nucleoside analogs, their methods of synthesis and therapeutic use that have been the subject of recent patent applications. Therefore, the main areas of focus were synthetic procedures for the preparation of selected fluorinated nucleosides such as anticancer clofarabine, gem-fluorinated 5-azacytidine analogs and ^{18}F -labeled agents FLT and FMAU used in the clinic as PET tracers. As the synthesis of ^{18}F -labeled nucleoside-type agents is challenging, over the past years a significant effort was made to develop improved effective strategies for the preparation of such agents. Herein, we also outlined current developments and processes for the preparation of blockbuster anti-HCV drug sofosbuvir, which may be considered industrially amenable. In addition, we emphasized examples of patent inventions directed towards synthesis and biological applications of phosphoramidate prodrugs based on fluorinated-nucleosides. A number of other therapeutic applications of fluorine-containing nucleosides are highlighted with modified RNAi agents containing 2'-fluoro or 2'-methoxy-xylose analogs as potential modulators of gene expression or novel fluorinated deoxyribo-cyclic dinucleotides (CDNs) as activators of receptors of STING signalling pathway. Due to the enhanced pharmacokinetic and pharmacodynamics profiles that fluorine can induce in drug-like molecules, the substitution of structural hydrogen atoms with fluorine will continuously be applied in medicinal chemistry programs as a powerful tool to improve key drug-like features. As the nucleos(t)ide analogs are considered as cornerstone in the treatment of viral and cancer diseases, and there is a continuous need for novel and more effective agents, further modifications of nucleosides including fluorination are likely to continue and remain a rich source of patent literature.

Executive summary:

- Insertion of fluorine in the structures of established bioactive nucleosides has resulted in the approval of several new drugs with both antiviral and anticancer therapeutic scopes.

- The fluorine atom, with its unique characteristics, has a pivotal role to alter a variety of physicochemical properties of drug-like molecules, thus influencing their molecular stability and target binding properties.
- Recent patent applications on fluorine incorporation chemistry using nucleophilic fluoride have greatly influenced the continuously evolving ^{18}F -PET radiochemistry.
- Examples of recent patent applications disclosing improved methods for the preparation of anticancer fluorine nucleosides and prodrugs are discussed within the anticancer nucleosides section.
- Examples of recent patent applications reporting on improved/alternative methods for the preparation of anti-HCV agent sofosbuvir and novel acyclic nucleoside phosphonates are discussed within the antiviral nucleosides section.
- Examples of fluorinated nucleosides with miscellaneous uses and methods for their preparation based on the recent patent applications are provided in this review.

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